STANDARDISATION OF TECHNIQUES FOR THE MEASUREMENTS OF TRITIUM (OBT AND TFWT) IN ENVIRONMENTAL MATRICES AROUND PHWR NUCLEAR POWER PLANT

Synopsis of the Thesis to be Submitted to the Mangalore University for the Award of the Degree of Doctor of Philosophy in Physics

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1.0 Introduction

Tritium (^{3}H), a radioactive isotope ($T_{1/2}$=12.3 y) of hydrogen and it decays to ^{3}He with average beta energy of 5.7 keV (Singh et al., 2010, ICRP 1983, Okada and Momoshima 1993, Korolevych and Kim 2013, Chae et al., 2011). It is produced by nuclear reactions that occur naturally in the environment due to the interaction of high energy cosmic rays with oxygen and nitrogen atoms in the upper atmosphere (Popoaca et al., 2014). The estimated production rate is 1.48×10^{8} GBq a^{-1}, resulting in a steady-state natural inventory of 2.59×10^{9} GBq in the world (IAEA, 2004). In addition to natural sources, small quantities of tritium may be released into the environment during the regular operation of the nuclear power plants (NPPs) (IAEA, 2010) and other nuclear facilities such as fusion reactors, fuel reprocessing plants, and heavy water production facilities. In a nuclear power plant, tritium is produced during the fission of heavy nuclei and by the interaction of neutrons with coolants, moderators, and light elements such as lithium, beryllium, and boron. Among different types of NPPs, pressurised heavy water reactors (CANDU type) are the main sources of anthropogenic tritium, with an estimated discharge rate of 3.70×10^{3} GBq $GW(e)^{-1}$ a^{-1} and 2.59×10^{4} GBq $GW(e)^{-1}$ a^{-1} through gaseous and liquid effluent respectively (IAEA, 2004).

Being an isotope of hydrogen, tritium in the environment is present as tritiated gas (HT) and tritiated water (HTO). In environmental biota, tritium exists in two forms: (i)tissue free water tritium (TFWT), and (ii) bound to atoms in the material as organically bound tritium (OBT) (Belot and Guenot, 1984). The OBT can further be distinguished as exchangeable OBT (E-OBT) which is bound to nitrogen, oxygen or sulphur atom, and non-exchangeable OBT (NE-OBT) which is bound to carbon atom via stable covalent bonds.

Following an intake of tritium by animals and uptake by plants, a fraction of the tritium may become incorporated into organic molecules such as carbohydrates, fats and proteins to form NE-OBT. While both forms, i.e. E-OBT and NE-OBT, can be regarded as OBT, it is the non-exchangeable OBT which has longer retention time in the body- of few weeks, and its elimination depends on the metabolism of organic molecule involved (IRSN, 2010; Boyer et al., 2009; Kim et al., 2008, 2013). On the other hand, TFWT has a retention period of a few days in the body.

2.0 Motivation for this study

The importance of OBT was realised only within the last decade, and studies are undertaken to understand its behaviour and develop analytical methods, which are essential to assess tritium distribution in the environment and dose consequences (Baglan et al., 2018). But to monitor the tritium integration in environmental matrices, the sensitivity and accuracy of the analytical procedure are of primary importance. Therefore, to analyse the tritium fractions in an environmental sample, a suitable analytical procedure needs to be developed with a specific emphasis on avoiding possible contamination during critical stages (Baglan et al., 2005). Realising the importance of OBT measurement, efforts are being made by various laboratories worldwide to develop improved analytical methods (Osvath et al., 2016). The Commission for the Establishment of Methods of Analysis (CETAMA), France, organised an inter-comparison program during in 2009-2010 for the measurement of environmental levels of OBT in grass samples. A few more such programs followed this inter-comparison exercise; with the distribution of sediment sample by Geosciences Advisory Unit (GAU), Southampton University, United Kingdom; wheat (*Triticum aestivum*) sample by Atomic Energy and Alternative Energies Commission (CEA), France, and fish sample by Canadian Nuclear Laboratories (CNL), Canada in the year 2014, 2015 and 2018, respectively. Since the publication of the CETAMA 384 method (2013), the XP M60-824 standard (2016) (Baglan et

al., 2018) and the NF M60-824 PR standard (2020) (NRD, 2020), OBT measurements are included in environmental monitoring programs in France, Canada, and Romania. The inter-comparison exercises conducted for OBT measurements have brought out the fact that the methods for NE-OBT determination are still not harmonised, and there is scope for further improvement.

While there are studies reported on NE-OBT for temperate regions, studies for tropical monsoonal climatic regions are sparse. Monitoring of tritium in the gaseous and aquatic environment is carried out routinely around nuclear power plants (NPPs) worldwide, but measurement of OBT activity in environmental matrices was not considered as mandatory in a majority of the countries. This is because the measurement is a time-consuming process due to the elaborate chemical treatment procedure for the sample and requires specialised instruments for processing the samples. Instead, the TFWT activity is measured, and from this, the OBT activity in terms of Bq kg^{-1} fresh mass is measured using the generalised value of water equivalent factor (WEQ_p).So far, on a global scenario, experimental data reported on this parameter are minimal. Generating an experimental database on WEQ_p for different types of biota samples of both tropical and temperate regions is essential to validate the estimated data to ensure accurate determination of OBT activity and dose assessments to the public.

3.0 Aims and objectives

The aims and objectives of the present study are:

i. standardisation of a batch thermal combustion method using a tube furnace oxidation system (pyrolyser) and liquid scintillation counter (LSC) for NE-OBT determination in environmental matrices, and its validation through international inter-comparison exercises,

ii. to generate a reliable database on water equivalent factor (WEQ_p) for the terrestrial and aquatic biota of the tropical monsoonal climatic region of Indian sub-continent which will be immensely useful for the quantification of NE-OBT activity in biota samples,

iii. to conduct a long term study and to generate a reliable database on NE-OBT activity in different environmental matrices in the vicinity of a pressurised heavy water reactor (PHWR) NPP at Kaiga, India

iv. to evaluate the NE-OBT to TFWT activity ratio for different plant species, and

v. to quantify the dose to the public due to the ingestion of various forms of tritium originated from the Kaiga NPP.

4.0 Overview of the work done

4.1 Study area

Kaiga (Latitude: 14˚ 51’ 55.2” N, Longitude: 74˚ 26’ 22.7” E) is situated about 58 km east of the coastal town of Karwar, Karnataka, where four pressurised heavy water reactor (PHWR) are in operation. Kaiga NPP commonly known as Kaiga Generating Station (KGS) is in operation since November 2000. Each reactor has a capacity of producing 220 MWe of electricity with a total installed capacity of 880 MWe. Kaiga is adjoined to river Kali and is situated in the valley of Western Ghats of Karnataka. The landscape of Kaiga is unique, surrounded by hills (altitude ranging from 40 m to 600 m from the mean sea level) and dense evergreen, deciduous forest comprising of hundreds of different vegetation species, belonging to different taxonomic groups, typically found in the Western Ghats. Due to hills and dense forest surrounding it, Kaiga manifests unique meteorological and ecological characteristics. This geographical feature in the vicinity of the Kaiga has a unique quality of acting like a trapping bowl for aerosols and also for the fallout radionuclides (Karunakara et al., 2001).

The soil in the study area is predominantly lateritic, dark brown, loamy textured with fine to medium grains.

The South West Coast region of India is influenced by two types of air masses– continental and oceanic. The Arabian Sea has a unique weather pattern because of the Indian monsoon and the associated winds that reverse the direction seasonally. Kaiga receives an average rainfall of 3800 mm y^{-1} from June to September, and the dominant wind direction in the study area is easterly winds during winter and westerly/ southwesterly during rainy and summer seasons, with respect to the nuclear power plant. About 95% of rainfall is received during a period of six months, from May to October, and the region generally experiences a dry spell from December to March. The annual ambient temperature in Kaiga varies from 19 to 35 °C, the lower value corresponds to the early morning hours of the winter season, and the higher value corresponds to the early afternoon hours of summer.

4.2 Control location

For a comparison of the results on NE-OBT activity in the vicinity of NPP with a clean air region (with minimal or no anthropogenic source of tritium), two locations were identified as control sites. They are (i) Beltangady (12.9887◦ N, 75.2725◦ E), and (ii) Karkala (13.2151◦ N, 74.9962◦ E), both situated in the Western Ghats at an aerial distance of about 300 km from Kaiga. The climatic conditions for these sites are similar to that of Kaiga.

4.3 Details on sampling locations

The sampling locations were identified in different sectors in the vicinity of the NPP, and these were distributed in the 2.3-5 km, 5-10 km, and 10-20 km radius zones of the NPP with NPP being the common centre for each zone. Since reserved forest exists throughout the region, all the sectors in the vicinity of the NPP could not be covered for the study due to poor approachability with no road connectivity.

4.4 Sample collection

The samples were collected for three consecutive years: 2015, 2016 and 2017; during summer (March-May), autumn (June - September), and winter (October - February). Same species were collected from every sampling location and during different seasons, which helped to study short term and long term variations. The typical samples collected were the leaves from *Mangifera indica* L. (Mango), *Tectona grandis* L.f. (Teak), *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn (Terminalia) and *Chromolaena odorata* (L.) R.M. King & H. Rob *(*Eupatorium odoratum). Fruits and vegetables that are grown by the farmers residing in the sampling locations were collected. Paddy, which is the staple food of the people residing in the vicinity of the NPP, was collected during the time of harvest. Fish samples of the local variety, freshwater fish- Rohu (*Labeo rohita*); and saltwater fish-Mackerels (*Rastrelliger kanagurta*), and Sardine (*Sardina pilchardus*) were collected from the local fishermen who undertake fishing in the river and other water bodies in the vicinity of the Kaiga.

4.5 Processing of the samples for NE-OBT and TFWT determination

After collection, the samples were sealed in polythene bags and were frozen in a freezer so that there was no contact with the surrounding atmosphere, until the time of analysis. The collected samples were analysed within 36 hours of sample collection. At the time of analysis, the samples were taken out of the freezer and divided into two equal parts. One part of the sample was subjected to NE-OBT analysis and the other part for TFWT analysis.

The pre-treatment of samples for NE-OBT analysis consisted of drying and grinding the samples for homogenisation. The samples were dried in an oven at 90 °C to remove all the water content and acquire a constant dry mass. The dried samples were then subjected to E-OBT elimination and NE-OBT determination.

4.6 TFWT measurement

Two predominant plant species of south-west coast region of India: *Mangifera indica* L. and *Chromolaena odorata* (L.) R.M. King & H. Robwere studied for TFWT activity. The samples were cut into small pieces for efficient lyophilisation process. The sample was lyophilized for 5 to 6 hours to extract water from the sample.

About 10 mL of extracted water was mixed with 10 mL of Ultima Gold uLLT scintillator in a low diffusion polyethylene LSC vials (PerkinElmer, USA), stored in the dark to reduce chemiluminescence. The sample was counted in Quantulus 1220LSC for two cycles of 100 min each (Nayak et al., 2019).

4.7 Instruments used for the determination of NE-OBT

Several laboratories around the world are working on the development of an efficient method to determine NE-OBT in various environmental compartments. Numerous techniques have been explored to facilitate tritium extraction from a wide range of sample matrices including tube-based combustion furnaces (catalytic furnaces) such as the Raddec Pyrolyser-6 Trio (Kim et al., 2008), automated Sample Oxidisers 307 (PerkinElmer), Zinsser Ox-501 and Parr Oxygen Bombs (Yankovich et al., 2011). All these techniques further require LSC for activity measurement.

4.7.1 Pyrolyser

The pyrolyser system (model-Pyrolyser 6 Trio^{TM}, Raddec International Ltd, Southampton, UK) was used in the present study for the combustion of samples. The instrument comprises of six quartz tubes for batch combustion of samples that pass through three contiguous furnaces, each holding 30 mm silica glass work tubes that narrow at one end to accommodate the catalyst pellets. The pyrolyser system is divided into three different zones, namely, the sample-zone furnace (35-900℃), mid-zone furnace (500-900℃), and

catalyst-zone furnace (600-900℃). Air from a compressor (~ 1 bar) and oxygen (~ 1 bar) are introduced into quartz glass work tubes to aid combustion. The combustion products from the sample are passed through the catalyst zone, which consists of 10 g of 0.5% Pt- Alumina catalyst, which is placed between the quartz wool. The use of catalyst facilitates the complete oxidation of the combustion products. The tritiated water produced from the combustion was collected in glass tubes maintained at -110 °C in the cryocooler.

There are eight pre-defined programs such as NORMAL, RAPID, ORGANIC, FISH, WET ASBESTOS, DRY ASBESTOS, GRAPHITE and BIOCONC for combusting the sample in the pyrolyser system. In addition to these, a user can develop programs depending upon specific requirements. Each pre-defined program differs in temperature, ramp, and hold time. The program can be selected based on the type of sample to be combusted (Lim et al., 2014).

4.7.2 Quantulus 1220 liquid scintillation counter

Quantulus 1220 (PerkinElmer, USA) ultra-low background LSC was used for NE-OBT measurements. The graphical user interface is a menu-driven software program, WinQ (1220-307). This program offers an unlimited number of parameter groups and protocols for the analysis of the samples. It also provides a live display of selected spectra on the computer screen. Raw data saved on the computer can later be processed off-line with EASY View spectrum analysis software which displays up to six spectra simultaneously.

4.8 Optimisation of the mass of samples for combustion for WEQ_P and NE-OBT determination

To evaluate the optimum mass of vegetation samples that can be combusted efficiently dried leaf samples of *Chromolaena odorata* (L.) R.M. King & H. Rob were combusted in pyrolyser selecting the pre-defined ORGANIC program. The quartz pyrolyser

boats were filled with finely powdered sample and were combusted to determine the mass of the combustion water that was collected in a glass tube placed in cryocooler at the end of the combustion program. Similarly, the finely sieved sediment sample was filled in quartz pyrolyser boats and combusted selecting the pre-defined NORMAL program, and the fish sample was combusted using FISH programs.

4.9 Optimisation of parameters for the measurement of NE-OBT using LSC

The overall performance of the LSC technique can be assessed by studying parameters like counting efficiency, intrinsic background contribution, quench resistance, sample stability over time, sample load capacity, and sample compatibility. These factors must be accurately quantified to yield accurate results during routine measurements.

4.9.1 Optimisation of sample to scintillator ratio

To optimise water to scintillator ratio, ultrapure water and scintillator (Ultima Gold uLLT) were mixed in 20 combinations of water + scintillator (1 mL+19 mL, 2 mL+18 mL, etc.) in separate low-diffusion polyethylene vials and the miscibility of the mixture was checked. It was observed that 10 mL of the scintillator was miscible with 10 mL of water without any phase separation and turbidity formation. Hence, 10 mL+10mL combinations of sample + scintillator were used for all the optimisation experiments.

4.9.2 Optimisation of counting sensitivity

The performance of the LSC was quantified by determining its signal to noise ratio, which was obtained from statistical considerations and expressed as a function of efficiency and background and is termed as the figure of merit (FOM). The FOM values were calculated using the Eq. as follows:

$$\mathrm{FOM} = \frac{\mathrm{E}^2}{\mathrm{B}}$$

where E is the counting efficiency, and B is the background count rate.

For Quantulus 1220 used in the present study, the optimum window range acquired was50-170, and the corresponding FOM value was determined to be 620.

4.9.3 Generation of quench curve for efficiency determination

The quench curve was established by preparing a set of tritium standards in which absolute activity (dpm) per vial was constant, but quench was increased from vial to vial. In general, the quench standards with known tritium activity were prepared by maintaining the mass constant and adding external quench, i.e. carbon tetrachloride (CCl_4). These quench standards were counted in LSC for 600 s. The efficiency was calculated based on the knowledge on the activity added, and from the beta spectrum. The efficiency was plotted against SQP(E) values, and a second-order polynomial curve was fitted to data points.

However, it was observed that the SQP(E) values for this set of quench standards ranged from 630 to 700. On the other hand, environmental samples combusted in pyrolyser had a wider range of SQP(E) values. Hence, quench curve was generated by varying the mass of both water and scintillator in 15 vials and maintaining the total mass as 20 mL (e.g., 1 mL of water + 19 mL of the scintillator, 2 mL water +18 mL scintillator, 3 mL water+ 17 mL scintillator, 4 mL water+ 16 mL scintillator, …. 15 mL water + 5 mL scintillator). In this case, no external quench agent was added, since different volumes of scintillator resulted in varying quench levels. The vials were spiked with known tritium activity and counted in LSC to determine the efficiency. The efficiency was plotted against SQP(E) values, and a second-order polynomial curve was fitted to data points.

4.9.4 Determination of minimum detectable activity

The MDA for TFWT was calculated for the combination of 10 mL of ultra-pure water with 10 mL of Ultima Gold uLLT scintillator for a counting time of 12000 s in the LSC. The MDA for NE-OBT was calculated for samples combusted using pyrolyser and counted using

LSC. For determination of MDA, leaf samples of *Chromolaena odorata (L.)* R.M. King & H. Rob were collected from a clean air region processed and combusted using the ORGANIC program. Similarly, the MDA for fish samples was determined by combusting *Rastrelliger kanagurta* collected from a clean air region. From the counts obtained, MDA (Bq kg^{-1} w.r.t. dry mass or Bq L^{-1} of combustion water) at 95% confidence level was calculated.

4.10 Validation of the results through inter-comparison exercises

Validation of the method employed in the present study was performed by participating in the international inter-laboratory inter-comparison exercises. The sediment samples distributed by Geosciences Advisory Unit (GAU), Southampton University, UK, wheat sample distributed by Atomic Energy and Alternative Energies Commission (CEA), France, and fish sample distributed by Canadian Nuclear Laboratories (CNL), Canada in the year 2014, 2015 and 2018, respectively, were used for the validation of the method (Nayak et al., 2019). The mean OBT activity determined by the combustion of the sediment sample was 148.2 ± 13.2 Bq kg^{-1} (w.r.t. dry mass). The OBT activity value assigned for this sample was 168.0 ± 22.0 Bq kg^{-1} (w.r.t. dry mass). The results reported by participating laboratories were accepted if they were in the range of 130–210 Bq kg^{-1} (w.r.t. dry mass). The mean OBT activity measured by the combustion of the wheat sample was 38.0 ± 4.2 Bq kg^{-1} which agreed well with the target value of 41.1 ± 1.8 Bq kg^{-1} (both w.r.t. dry mass) (Baglan et al., 2018). The limits for validation of results reported by participating laboratories were in the range of 29–58 Bq kg^{-1} (w.r.t. dry mass). For the fish sample, the mean OBT activity obtained was 20.4 ± 7.0 Bq kg^{-1} (w.r.t. dry mass) against the assigned value of 24.2 ± 7.0 Bq kg^{-1} (w.r. t. dry mass). The results submitted by the participating laboratory were considered acceptable if the reported OBT values were within ±15 Bq kg^{-1} (w.r.t. dry mass) from the assigned value.

Repeatability in the measurement was ascertained by combusting subsamples of wheat, sediment and fish (mentioned just above). For the five trials for each of the above three matrices, the obtained OBT value varied in a narrow range with deviation from target values was in the range 2–17%, 6–16% and 5–28% for wheat, sediment and fish, respectively with corresponding mean values of 8.7%, 11.7%, and 17.9%. The higher deviation from the target value in the case of fish was due to the lower mass of the sample taken for combustion (2 g) as against the wheat (4 g) and sediment (8 g).

4.11 Evaluation of degradation of the catalyst due to the combustion of the fish sample

To study the degradation of the catalyst, the wheat sample with known OBT activity of 41.1 Bq kg^{-1} w.r.t. dry mass, used in the worldwide inter-laboratory inter-comparison exercise conducted in the year 2016 by CEA (Baglan et al. 2018), was combusted using a virgin catalyst, and activity was determined. After this, the fish sample was combusted with the same catalyst, and this was followed by the combustion of wheat samples again. The OBT activity was determined for all three experiments. It was observed that the recovery of the OBT from the wheat combusted after combusting fish samples decreased by ~50%. Hence, the catalyst used during combustion of high fat and lipid content samples needs to be replaced before combusting subsequent samples, whereas in the case of terrestrial biota samples of plant origin, soil, sediment, etc. (which have low fat and lipid contents), the catalyst can be used for combustion of about 20 samples.

4.12 Uncertainty in the determination of WEQ_p and NE-OBT activity

The uncertainty associated with the method was computed by propagating the relative error associated with (i) weighing of the sample taken for analysis (u_1), (ii) determining the counting efficiency of LSC (u_2), (iii) count rate of the sample (u_3), (iv) determining NE-OBT

recovery from the sample (u_4), and (v) determination the WEQ_p. Error propagation was performed as under (Kessler, 2015):

$$u = \sqrt{u_1^{\,2} + u_2^{\,2} + u_3^{\,2} + u_4^{\,2} + u_5^{2}}$$

The error associated with the determination of sample mass (u_1) was calculated by counting the same sample 100 times. The error associated with counting efficiency was calculated by counting a quench standard (5 times) (u_2). The error associated with the count rate of the sample (u_3) was determined by counting the same sample 20 times.The error associated with the recovery of NE-OBT from the sample during the combustion process (u_4) was determined by analysing several subsamples of wheat, sediment and fish, distributed during international inter-laboratory inter-comparison exercise. The error associated with the recovery of hydrogen species was determined by combusting subsamples of *Triticum aestivum* L. and fish sample. With these, the overall uncertainty in the measurement was determined to be 11.8 % (Nayak et al., 2019).

5.0 Important finding and conclusions drawn from the study

Optimisation of a batch combustion method for NE-OBT determination

- A batch combustion method using a tube furnace system (pyrolyser) and determination of the NE-OBT in the combustion water by LSC has been optimised for the terrestrial and aquatic biota samples.
- The combustion programs and the temperature profiles for efficient combustion of different types of sample matrices were studied. For vegetation samples with high organic content, the ORGANIC program (max temperature = 500 °C, ramp= 3 °C min^{-1}, hold = 20 min) was capable of efficient combustion of the samples with optimum tritium recovery.

- For sediment and soil samples, which contain higher mineral contents when compared to the terrestrial biota samples, the NORMAL program was better suited for effective extraction of tritium. The temperature profile of this programme (with temperature increasing up to 600 °C, ramp= 3 °C min^{-1}, hold = 20 min) helped in efficient breaking down of minerals for tritium desorption.
- Combustion of samples with high-fat content, such as fish is quite challenging and efficient combustion of fat content requires high temperature. FISH program with the endpoint temperature of 800 °C was efficient in extracting tritium from sample matrices.
- A maximum of ~4 g of the terrestrial biota samples such as wild plant leaves, fruits and vegetables, ~8 g of soil/sediment sample and ~2 g of fish sample can be combusted efficiently in the pyrolyser tube furnace system for the determination of NE-OBT activity in the combustion water.
- The minimum detectable activity (MDA) of NE-OBT in the terrestrial biota samples of plant origin combusted in pyrolyser and counted in Quantulus-1220 LSC in the tritium energy window of 50-170 channels was 5.2 Bq kg^{-1} (dry mass) which corresponds to 8.6 Bq $L^{-1}$of combustion water, for a counting time of 12,000 s. Similarly, the MDA achieved for the fish samples was 9.9 Bq kg^{-1} (dry mass) which corresponds to 15.3 Bq L^{-1} of combustion water,for a counting time of 12,000 s.
- Repeatability in the measurement was ascertained by combusting the subsamples of wheat, sediment and fishobtained from international inter-laboratory inter-comparison exercises. The measured NE-OBT activity in these samples varied in a narrow range, and the deviations from the target values were the range 1-17% (mean values of 9.3%) for wheat, 6-16% (mean values of 11.7%) for sediment samples, and 5-28% (mean values of 17.9%) forfish samples, respectively. Excellent repeatability in NE-OBT measurement

was achieved for the method optimised in the present work, with deviations of the individual measurements were within ±2 σ from the target value.

- Participation in the inter-laboratory inter-comparison exercises demonstrated that the method optimised in the present study is capable of yielding accurate results.
- The fresh batch of catalyst loaded to the tube furnace system for the oxidation of the sample can be used to combust up to 20 batches of samples when terrestrial biota samples of plant origin, soil and sediment samples are combusted. However, the catalyst used during the combustion of high-fat and lipid content samplessuch as fish, milk, or plasticscannot be used for subsequent combustions because of severe degradation.

WEQ_p factors for terrestrial and aquatic biota

- This is the first detailed study on WEQ_p factor undertaken on a global scenario, providing a robust experimental database for various terrestrial biota samples representing the tropical monsoonal climatic region.
- WEQ_p varied within a very narrow range of 0.473-0.633 L kg^{-1} with a mean value and the associated standard deviation of 0.562±0.011 L kg^{-1} for fruit samples (N=80). For vegetables, it varied in 0.492-0.678 L kg^{-1} with a mean value and the associated standard deviation of 0.570±0.034 L kg^{-1}(N=100).
- WEQ_p values for different terrestrial biota samples (leafy and non-leafy vegetables, fruits, nuts, grains and root crops) were similar, and the difference between the mean values of each category of samples was <4%.
- The mean value and the associated standard deviation of WEQ_p for the fish sample was 0.695±0.019 L kg^{-1} (N=16), which was 1.25 times higher than that for terrestrial biota samples. The higher WEQ_p value in fish was due to high-fat content.
- A comparison of the WEQ_p values calculated based on the knowledge of the hydrogen content in protein, fat and carbohydrates, and those derived experimentally revealed that

for all types of terrestrial and aquatic biota, the deviations between the two entities are generally <10%.

- However, the deviation between measured and calculated WEQ_p values was higher in the case of nuts. For *Cocos nucifera* L., the deviation was 42.8%, and for *Areca catechu* L., it was 16.3%. This may be due to the high-fat content (up to 41% in the case of *Cocos nucifera* L.) in these types of samples. The combustion program (ORGANIC) adopted in this study may not be suitable for the complete oxidation of hydrogen from the fat molecule. A modified temperature profile for the combustion needs to be explored in this case.
- Linear regression performed between the calculated and experimental WEQ_p values yielded a statistically significant positive correlation of 0.81, and slope =0.97 for 294 samples of 29 species. This confirmed a good agreement between the calculated and experimental values.
- The WEQ_p data generated experimentally in the present study were compared with those compiled in IAEA (2010), which are derived through calculations based on the knowledge on the hydrogen contents of protein, fat and carbohydrate. The deviations between the generalised data presented in IAEA (2010) and the present study were 7.5%, 9.8%, 3.8% and 6.1% for non-leafy vegetables, leafy vegetables, root crops, and fishes, respectively. Such deviation is expected because the results presented in this work represent experimental data for the samples from the typical tropical monsoonal climatic region. In contrast, those listed in the IAEA report are for temperate countries.
- Although the maximum deviation between the calculated and the experimental datasets of WEQ_p is <10% for most food matrices, it cannot be ignored for accurate radiation dose assessments in the vicinity of the nuclear facilities.

- The data set generated in the present study is based on analysis of a significantly large number of samples, and hence, statistically robust. It will serve as a crucial reference database for NE-OBT studies and accurate dose assessments.

NE-OBT activity in fruits and vegetables

- This is perhaps the first comprehensive study on NE-OBT activity in the Asia Oceanic region. A large number of samples were analysed, and a robust database has been established.
- The NE-OBT activity in fruits in the vicinity of the Kaiga NPP (2.3-20 km zone) varied between 10.1-63.8 Bq L^{-1} (N=105) with the mean value and the associated standard deviation of 25.7±11.7 Bq L^{-1}. In vegetables, it varied in the range of 9.8-61.7 Bq L^{-1} (N=81) with a mean value and the associated standard deviation of 27.1±13.7 Bq L^{-1}.
- The NE-OBT activity in fruits and vegetables decreased with the increase in the distance between the sampling station and the NPP.
- The statistical analysis of the NE-OBT dataset for the samples collected from the 2.3–20 km zone of the NPP revealed that the activity in 78.9% of the samples (N=147) was less than <40.0 Bq L^{-1}, and only 39 samples exhibited activity >40.0 BqL^{-1}.
- The NE-OBT activity values for the samples analysed from the clean air region varied from <8.6-14.5 Bq $L^{-1}$with a mean value and the associated standard deviation of 11.5±1.8 Bq L^{-1} in fruits (N=32), and from <8.6-14.3 Bq $L^{-1}$with a mean value and the associated standard deviation of 11.3±1.6 Bq L^{-1}in vegetables (N=22).
- Statistical analysis of the data proved that the NE-OBT activity in terrestrial biota was similar during all the seasons of the year.
- The radiation dose due to the ingestion of the NE-OBT through vegetables, to the public in the 2.3-20 km zone of Kaiga NPP was calculated to be 0.0029 μSv y^{-1}. This constitutes

0.0003% of the annual dose limit (1000 μSv y^{-1}) recommended by ICRP to the general public.

- The radiation dose due to intake of tritium through food, water, and air, for the public in the 2.3-20 km zone of the Kaiga NPP was estimated to be 0.293 μSvy^{-1}, which is 0.03% of the annual dose limit of 1000 μSv y^{-1} for the general public recommended by ICRP.
- The NE-OBT activity observed in the vicinity of Kaiga NPP is similar to those reported for other parts of the world.

TFWT and NE-OBT activity in plants

- This is the first detailed study on NE-OBT and TFWT in wild plant leaves in the environment of the NPP situated in the tropical monsoonal climatic region. This study has established a comprehensive database on two important forms of tritium in the terrestrial plants.
- The analysis of 289 samples from the vicinity of Kaiga NPP showed that the mean values and the corresponding standard deviation of NE-OBT activitywere 37.5±11.6 Bq L^{-1}, 24.9±5.8 Bq L^{-1}, 13.2±3.1 Bq L^{-1} for 2.3-5 km, 5-10 km and 10-20 km zones, respectively. Similarly, the mean value and the associated standard deviation of TFWT activity were 40.8±10.6 Bq L^{-1}(N=24), 31.1±4.7 Bq L^{-1}(N=32) and 20.1±6.7 Bq L^{-1} (N=24) for 2.3-5 km, 5-10 km and 10-20 km zone. In general, the NE-OBT and TFWT activity decreased as the distance between the sampling station and the NPP increased. The NE-OBT activity in 2.3-5 km zone was 1.5 times higher than 5-10 km zone and 2.8 times higher than 10-20 km zone. Similarly, the mean value of TFWT activity in 2.3-5 km zone was 1.3 times higher than the 5-10 km zone and two times higher than the 10-20 km zone.
- The NE-OBT activity in the clean air region, situated ~300 km away from the NPP ranged between <8.6-14 Bq L^{-1}(N=35) with a mean valueand the associated standard

deviation of 11.1±1.8Bq L^{-1}, and the TFWT activity varied between 4.5-8.2 Bq L^{-1}(N=12) with a mean value and the associated standard deviation of 6.1±1.3 Bq L^{-1},

- Statistical analysis of the NE-OBT dataset revealed that 85.4% of the total number of samples analysed (N=289) had activity ranging between 10-35 Bq L^{-1}, and 14.5% of the samples (42 samples) had activity higher than 35 Bq L^{-1}. Similarly, the analysis of the TFWT dataset showed that 85% of the total number of samples (N=68) were within 40 Bq L^{-1}, and only 12 samples had activity greater than 40 Bq L^{-1}.
- This study has revealed that the NE-OBT integration into leaves is not species-dependent. On the other hand, environmental conditions, such as climate, soil nature and exposition to light may be the controlling parameters for NE-OBT incorporation into plants.
- Monitoring of NE-OBT for three consecutive yearsduring summer season resulted in mean activity values and the associated standard deviation of 24.9±13.2 Bq L^{-1}, 25.8±11.7 Bq L^{-1}, 25.3±10.9 Bq $L^{-1}$for the years 2015, 2016 and 2017, respectively. Similar observations were recorded for the winter seasons also. This points to a uniform release of tritium from the NPP.
- The statistical analysis of the NE-OBT activity dataset for the three seasons confirmed no significant variations in the activity with respect to seasons. The NE-OBT activity during the summer, autumn and winter seasons were 25.1±11.3 Bq L^{-1}, 25.1±12.6 Bq L^{-1}, and 24.9±10.3 Bq L^{-1}, respectively. The TFWT activity in the same order of seasons was 32.6±11.8 Bq L^{-1}, 29.3±8.9 Bq L^{-1}, and 28.4±10.1 Bq L^{-1}, respectively,
- The statistical analysis of the NE-OBT activity with the predominant wind direction confirmed that the dataset for the samples collected from the 5-10 km zone of the NPP exhibit a significant dependence on the wind direction and dependency was not observed for the samples collected from locations in the 2.3-5 km zone and 10-20 km zone. The diffusion of the tritium released through gaseous effluents in the close proximity of the

NPP may be a dominating factor governing the tritium activity in the 2.3-5 km zone. On the other hand, the 10-20 km zone is not affected by the tritium released through gaseous effluents. Hence, the NE-OBT activity is not expected to be influenced by the wind direction.

- The NE-OBT and TFWT activity ratio, R value, varied between 0.59-1.28, with the mean value of 0.82±0.22. This value of R, established from the measurement of a large number of samples, will immensely benefit the regular environmental assessment program of the NPP. One can measure the TFWT in the samples and estimate NE-OBT activity in terrestrial biota samples using the R value.
- This study has led to an important finding: the R value for the plants grown in the clean air region (1.89±0.41) was greater than that observed for the NPP's vicinity. This indicates that the plants might sequester tritium not only from air moisture but also from soil organic matter, and it could be a significant component. This necessitatesdetailed studies to understand the mechanism of NE-OBT partitioning in the terrestrial environment.
- Linear regression performed between the NE-OBT and TFWT activities in plants confirmed a statistically significant positive correlation coefficient of 0.72, for N=80, $p<<10^{-4}$.

6.0 Outline of the thesis

The thesis has been divided into six chapters, which includes the introduction chapter comprising of the detailed review of the previously reported studies on NE-OBT measurements. In chapter 2, the details of the methodology followed for the identification of sampling stations, sample collection, and processing are presented. This chapter also presents the details of the TFWT measurements.

A detailed description of the instruments used for combustion of the sample and measurement of NE-OBT and TFWT activity is presented in chapter 3. This chapter focuses on the optimisation of a method for NE-OBT measurement in various environmental matrices using the tube combustion system. Details are also presented on the mass of the terrestrial and aquatic biota sample taken for efficient combustion, the mass of the water produced from the combustion of these samples and the degradation of the catalyst during the combustion. Apart from this, optimisation of various parameters in the LSC counting, such as sample to scintillator ratio for NE-OBT measurements, generation and validation of the quench curve, determination of the figure of merit, the minimum detectable activity (MDA) of the method and evaluation of the uncertainty associated in the measurement are also discussed in this chapter. The methods optimised were validated through participation in international inter-laboratory inter-comparison exercises are also discussed in this chapter.

Chapter 4 presents the results and comprehensive discussion on the WEQ_p and NE-OBT activity in fruits and vegetable samples collected in the vicinity of the Kaiga NPP region. Extensive and robust experimental database on WEQ_p for terrestrial biota samples of plant origin collected from the vicinity of NPP at Kaiga and correlation between the experimental and calculated value is also discussed. Variations of NE-OBT activity with respect to different zones are presented in detail. Seasonal and temporal variations of NE-OBT activity are discussed. The chapter includes a brief discussion on the dose received by the population residing in the vicinity of the Kaiga NPP due to the ingestion of NE-OBT through different pathways and its comparison with reported values.

Chapter 5 presents the results, and comprehensive discussion on the NE-OBT and TFWT activity in the tropical wild plant leaves samples collected in the vicinity of the Kaiga NPP region. The data on NE-OBT activity in different leaves sample was subjected to various statistical tests and discussed. Seasonal, temporal, sectoral variation in NE-OBT

activity as well as with the wind directions, are discussed in detail in this chapter. The R value was derived and compared with international values.

Chapter 6 presents the summary and conclusions drawn from the study.

6.0 Research publications in peer-reviewed journals, National and International Conferences related to the thesis work

6.1 Peer-Reviewed Journals

1. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath S., Mohan M. P., Bharath, Trilochana Shetty, Sudeep Kumara K., Narayana B., Dileep B. N., Ravi P. M., Karunakara N. 2019. 'Organically Bound Tritium: Optimisation of Measurements in Environmental Matrices by Combustion Method and Liquid Scintillation Spectrometry'. J. Radioanal Nucl. Chem. 319, 917-926.
2. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath S., Mohan M. P., Bharath, Trilochana Shetty, Sudeep Kumara K., Narayana B., Dileep B.N., RaviP.M., Karunakara N. 2021. 'Determination of organically bound Tritium concentration in fish by thermal oxidation and liquid scintillation counting'. Health Phys. 120(1), 1-8.
3. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath S., Mohan M. P., Bharath, Ravi P. M., Karunakara N. Experimental database on water equivalent factor (WEQ_p) and organically bound tritium activity for tropical monsoonal climate region of South West Coast of India. Appl. Radiat. Isot. 166,109390.
4. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath S., Mohan M. P., Bharath, Ravi P. M., Karunakara N. Determination non-exchangeable organically bound tritium and tissue free water tritium in wild plants around Kaiga Nuclear Power Plant. J. Environ Radioact. (communicated).

6.2 International Conferences

1. **Rashmi Nayak S**., Shiny Renita D'Souza, Srinivas Kamath, Narayana B., Dileep B. N., Ravi P. M., and Karunakara N. 2017. 'Standardisation of methods for determination of OBT in environmental matrices using Pyrolyser'**.** 13th Biennial DAE-BRNS Symposium on Nuclear and Radiochemistry (NUCAR - 2017). PP: 660-662.
2. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath, Mohan M. P., Narayana B., Dileep B. N., Ravi P. M., and Karunakara N. 'Validation of the quench curve for the analysis of Organically Bound Tritium in environmental matrices by Liquid Scintillation Spectrometry'. 2018. Proceedings of International Conference on Developments towards Improvements of Radiological Surveillance at Nuclear Facilities and Environment (IARPIC - 2018). PP: 192.
3. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath, Mohan M. P., Narayana B., Dileep B. N., Ravi P. M., and Karunakara N. 2019. 'Determination of Water Equivalent Factor (WEQ_p) and Organically Bound Tritium concentration in Tropical climate'. 5th International Conference on Environmental Radioactivity (ENVIRA 2018). PP: 196.

6.3 National Conferences

1. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath, Mohan M. P., Narayana B., Dileep, B. N., Ravi P. M., and Karunakara N. 2017. 'Performance Evaluation of Automatic Sample Oxidation System for Organically Bound Tritium Determination in Environmental Matrices'. Proceedings of the National Conference on Radiation Physics (NCRP-2017). PP: 170-173.
2. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath, Mohan M. P., Narayana B., Dileep B. N., Ravi P. M., and Karunakara N. 'Performance Evaluation of Two Oxidation

Systems for Tritium Measurements in Environmental Matrices'. 2018. Proceedings of the 21st National Symposium on Radiation Protection (NSRP 21). PP: 112.

3. **Rashmi Nayak S**., Renita Shiny D'Souza, Srinivas Kamath, Mohan M. P., Narayana B., Dileep B. N., Ravi P. M., and Karunakara N. 2018 'Site Specific Data on Water Equivalent Factor for Determination of Organically Bound Tritium Concentration'. Proceedings of 20th National symposium on Environment (NSE-20). PP: 461-462.

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